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Tumor treatment by direct electric current. Tumor perfusion changes

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Abstract

A single-shot electrotherapy with 0.6 mA direct current for 60 minutes delivered via two needle electrodes placed subcutaneously on opposite sides of a tumor induced statistically significant tumor growth delay in SA-1 and LPB murine fibrosarcoma tumor models. A prolonged vascular occlusion is indicated by the results of staining tumors with a Patent Blue dye in the SA-1 tumor model immediately and 24 hours after one hour electrotherapy. The almost complete absence of tumor staining after treatment with electrotherapy in SA-1 tumors suggested that the tumor growth retardation could result from this prolonged vascular occlusion. However, statistically highly significant tumor growth delay in LPB tumors was accompanied by only a slight decrease of a tumor staining with Patent Blue. Thus, the observed effect of electrotherapy on both tumor growth retardation and perfusion in the LPB tumor model raises some doubt about the above hypothesis. © 1997 Elsevier Science S.A.

Keywords: Electrotherapy; Single-shot electrotherapy; Tumor

1. Introduction

It has been shown in several studies that direct electric current can be used successfully to treat solid malignancies. The antitumor effectiveness of electrotherapy has been demonstrated on various experimental tumor models [1–6] as well as in clinical trials [7,8]. In these studies electrotherapy was used as a local treatment. Electrotherapy has also been used as an adjuvant treatment to other therapies in order to potentiate their effectiveness [9,10]. Many attempts have been made to try to explain the antitumor effectiveness of electrotherapy alone or of its combined use with other therapies. Understanding of the mechanisms of antitumor action would be of extreme importance for optimizing existing and developing new treatment schedules.

Depending on electrode positioning with respect to the tumor, we account on different underlying mechanisms for the observed tumor growth retardation. When one or both of the electrodes are inserted in the tumor, the major part of the response is ascribed to cell killing due to large changes in pH in the vicinity of the electrodes. With appropriate spacing of multiple electrodes in the tumor, and with direct current of long duration, it is possible to eradicate most of the tumor mass [11]. However, if the electrodes are not placed in the tumor, but in its surroundings, similar tumor growth retardation is obtained at the currents used. Furthermore, in the 'field' configuration, where the electrodes are placed outside the tumor so that the tumor lies between the electrodes, neither temperature rise nor changes in pH in the tumor was found [12]. Tumor growth retardation in electrotherapy by low level direct current was also not correlated to the metal deposited from the electrodes [13]. However, it has been suggested that tumor growth delay in electrotherapy is due to vascular occlusion by damaging the vessels feeding the tumor at the locations of electrode insertion [14].

In our study we repeated electrotherapy in the field configuration, where the electrodes are not directly inserted in the tumor, on an additional tumor model. We also challenged the hypothesis that tumor growth delay observed in electrotherapy might be due to damaging vessels supplying the tumor.

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2. Experimental details

2.1. Animals and tumors

Female and male A/J strain mice were purchased from Rudjer Boškovič Institute, Zagreb, Croatia and C57 Bl/6 mice were purchased from C.E.R.J. animal facilities Laval, France. Animals were maintained at constant room temperature (24°C) in a conventional mouse colony. For the experiments, mice in good condition, without signs of fungal or other infection, 8–12 weeks old, 15–25 g weight were used. Fibrosarcoma SA-1 cells for tumor transplantation were obtained from the ascitic form of the tumor [15]. LPB fibrosarcoma cells were cultured in vitro [16]. Subcutaneous solid tumors were initiated by injection, dorsolaterally in the animals, of 5×10^5 viable SA-1 cells syngeneic to A/J mice or 5×10^5 LPB sarcoma cells [17] syngeneic to C57 Bl/6 mice. Animals inoculated with tumor cells were maintained in polycarbonate plastic cages 825 cm^2 until the tumors reached 100 mm^3 in volume (8–10 days). Subsequently they were marked individually and randomly divided into smaller groups subjected to a specific experimental protocol. Animals receiving the same treatment (experimental groups) were maintained together in smaller cages 363 cm^2 (6–8 per cage) and were fed ad libitum.

2.2. Electrodes and electrotherapy

Needle electrodes 1.0 mm in diameter and 20 mm long, with a spherical hub of Pt–Ir alloy (90–10%) were used. Electrodes were inserted subcutaneously parallel to each other for their whole length (spacing 20–22 mm) so that the tumor lay between them. Each of the electrodes was 5–8 mm away from the tumor edge. Electrodes were connected to the current source device designed to deliver a constant current (0.6 mA) by adjusting the voltage. This current level was chosen as a result of our previous studies [13]. Current and voltage were monitored throughout the treatment. At the start of electrotherapy the current was linearly raised from zero to the pre-set value over one minute and was similarly decreased to zero at the end of electrotherapy. Electrotherapy was performed when the tumors reached approximately. 100 mm^3 in volume as a single treatment and lasted one hour. Animals in control groups were treated in exactly the same way as animals subjected to electrotherapy except that no current was applied.

2.3. Assessment of electrotherapy effect on tumor growth

On the day of the therapy (day 0) and on each subsequent day, tumors were measured by calliper, and tumor volume was calculated using the formula $V = \Pi ab^2/6$, where a and b are two principal mutually orthogonal tumor diameters and $b \leq a$. For each experimental group mean volume and standard error of the mean was calculated on each day and presented as growth curves. For each individual tumor its doubling time (DT) was determined as time the tumor needed to double its initial volume (on day 0). Statistical significance of differences in tumor doubling time between controls and electrotherapy was evaluated by using the Mann–Whitney Rank–Sum test. Each of the experiments was repeated at least three times on each of the tumor models used.

2.4. Assessment of electrotherapy effect on tumor perfusion

Patent blue violet (Byk Gulden, Switzerland) was used in separate experiments of tumor growth to estimate tumor perfusion [18,19]. An 0.2 ml volume of saline solution of biological dye Patent blue violet (1.25%) was injected into the retroorbital sinus of animals after they were subjected to specific treatment. After the dye was left to distribute evenly through the tissues for approximately three minutes, animals were sacrificed and tumors were carefully removed. Tumors were than cut along their largest diameter and the percentage of stained versus non-stained cross-section was immediately estimated visually by two persons. The mean of both estimations was used as an indicator of tumor perfusion. The estimation of tumor perfusion was performed immediately and 24 hours after one hour of electrotherapy based on preliminary results at different time intervals after the start of electrotherapy as being representative of the effect of electrotherapy on tumor perfusion (data not presented). The results from different experiments were pooled together and were presented as mean and standard error of the mean for each experimental group. The difference between experimental groups was analyzed by using the Student t-test.

In our study Patent blue violet (PBV) was used instead of Evans blue, which is more widely used in dye perfusion studies. One of the reasons was that the colour contrast between stained and non-stained areas of tissue was far better with PBV. In addition, a pilot study demonstrated that both dyes produced very similar results in control tumors and in tumors subjected to electrotherapy (0.6 mA for 1 hour). Use of Patent blue and Evans blue resulted in $91 \pm 10\%$ (8) (mean $\pm$ standard deviation (sample size)) and $83 \pm 16\%$ (5) of stained cross-section area in control tumors respectively ($p = 0.261$) and in $13 \pm 11\%$ (8) and $12 \pm 8\%$ (6) respectively ($p = 0.884$) in tumors immediately after electrotherapy in SA-1 fibrosarcoma tumor model. Thus both dyes yielded comparable results in these preliminary experiments.

3. Results

3.1. Tumor growth

The single-shot electrotherapy with 0.6 mA direct current for 60 minutes delivered via two needle electrodes placed subcutaneously on opposite sides of the tumor

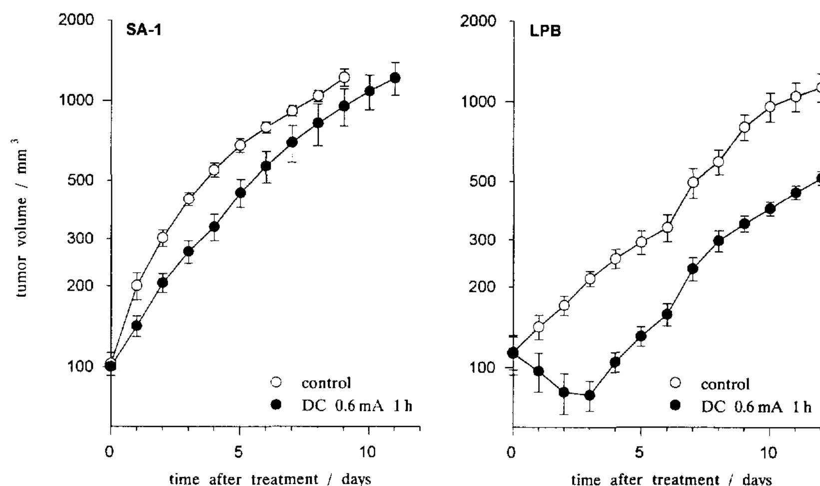

Fig. 1. Tumor growth following electrotherapy with 0.6 mA direct current of one hour duration on day 0 in comparison with control growth for SA-1 and LPB murine tumor models. For each experimental group mean volumes (dots) and standard errors of the mean (vertical bars) are presented ($n = 7$). Tumor growth was retarded in both tumor models used.

induced statistically significant tumor growth delay in both murine tumor models used. Induced tumor growth delay in SA-1 and LPB fibrosarcoma tumors is clearly shown in Fig. 1. In addition, the effect of electrotherapy on tumor growth also was estimated by calculating the mean tumor doubling time (DT/days) for each experimental group. The mean DT in SA-1 tumor model was 2.42 ± 1.54 (7) (mean $\pm$ standard deviation (sample size)) and 1.10 ± 0.37 (7) in electrotherapy and control experimental groups respectively ($p = 0.007$: Mann Whitney Rank Sum test). The mean DT in LPB tumor model was 7.25 ± 1.86 (7) and 3.59 ± 1.65 (7) in electrotherapy and control experimental groups respectively ($p = 0.004$: Mann Whitney Rank Sum test). Corresponding growth delays (GD/days) for SA-1 and LPB tumor models were therefore 1.32 ± 0.60 (mean $\pm$ standard deviation) and 3.66 ± 0.94 respectively.

3.2. *Tumor perfusion*

In the experiments where the effect of electrotherapy on perfusion was estimated by means of tumor staining, first the stained area of the untreated tumors was determined for both tumor models. Control tumors, of the same size but not subjected to electrotherapy, were $91 \pm 4\%$ (8) (mean $\pm$ standard deviation (sample size)) stained in the case of the SA-1 tumor model and $98 \pm 5\%$ (19) stained for the LPB tumor model. These results indicate that both tumors are well perfused at that stage of development and size. Immediately after one hour of electrotherapy by 0.6 mA, which in both tumor models resulted in significant tumor growth delay, SA-1 tumors were $13 \pm 11\%$ (7) stained whereas LPB tumors were $80 \pm 30\%$ (27) stained. Practically the same results were obtained 24 hours after electrotherapy was performed; SA-1 tumors being $26 \pm 23\%$ (8) and LPB tumors $81 \pm 35\%$ (8) stained. Staining of SA-1 tumors after electrotherapy was thus considerably lower than that of their controls ($p < 0.001$) but also considerably lower than staining of LPB tumors after the same treatment. LPB tumors subjected to electrotherapy were also less stained than their controls ($p = 0.013$), but staining was not as severely hindered as in the SA-1 tumors.

4. Discussion

Single-shot electrotherapy of one hour duration with a 0.6 mA direct current delivered via two needle electrodes inserted subcutaneously on opposite sides of the tumor retarded tumor growth in both murine tumor models used in our study. These results are in good agreement with our previous studies. Vascular occlusion indicated by the results of staining tumors with Patent Blue dye in the SA-1 tumor model immediately after one hour electrotherapy should persist for some time in order to produce an observable tumor growth retardation. Indeed, this was confirmed at 24 hours after electrotherapy. The almost complete absence of tumor SA-1 staining after they were treated with electrotherapy suggest that tumor growth retardation resulted from a prolonged vascular occlusion. However, the observed effect of electrotherapy on both tumor growth retardation and perfusion in the LPB tumor model raises some doubt about this hypothesis. Statistically highly significant tumor growth delay in LPB tumors was accompanied by only a slight decrease in tumor staining with Patent Blue.

Vascular damage due to electrotherapy is obvious, as estimated by Patent Blue in SA-1 tumor. Whether this damage is responsible for the observed growth delay remains to be seen by employing other techniques for tumor perfusion determination and by determining the dynamics i.e. the duration of the occlusion. It is also not clear

whether the observed difference in staining between control tumors and those subjected to electrotherapy is sufficient to explain the growth delay obtained in the LPB tumor model. Further studies are now being performed on oxygenation and perfusion status and dynamics of tumors and on the possible involvement of an immune response in the observed antitumor effect of electrotherapy.

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